Null Results in Brief

Lack of Replication of Seven Pancreatic Cancer Susceptibility Loci Identified in Two Asian Populations

Daniele Campa¹, Cosmeri Rizzato¹, Andrea S. Bauer³, Jens Werner², Gabriele Capurso³, Eithne Costello⁴, Renata Talar-Wojnarowska⁵, Krzysztof Jamroziak⁶, Raffaele Pezzilli⁷, Maria Gazouli⁸, Kay-Tee Khaw⁹, Timothy J. Key¹⁰, Franco Bambi¹¹, Beatrice Mohelnikova-Duchonova¹², Anette Heller¹², Stefano Landi¹³, Gianfranco Delle Fave¹⁰, John P. Neoptolemos¹⁰, Pavel Vodicka¹³, Renata Talar-Wojnarowska⁵, Krzysztof Jamroziak⁶, Raffaele Pezzilli⁷, Maria Gazouli⁸, Kay-Tee Khaw⁹, Timothy J. Key¹⁰, Franco Bambi¹¹, Beatrice Mohelnikova-Duchonova¹², Anette Heller¹², Stefano Landi¹³, Gianfranco Delle Fave¹⁰, John P. Neoptolemos¹⁰, Pavel Vodicka¹³, Markus W. Büchler¹, Nathalia Giese², and Federico Canzian¹

Abstract

Background: Two recent genome-wide association studies (GWAS) of pancreatic ductal adenocarcinoma (PDAC), conducted, respectively, in a Japanese and in a Chinese population, identified eight novel loci affecting PDAC risk.

Methods: We attempted to replicate the novel loci in a series of PDACs and healthy controls of European ancestry in the context of the newly formed PANcreatic Disease ReseArch (PANDoRA) consortium. We genotyped seven single-nucleotide polymorphisms (SNP): rs12413624, rs1547374, rs372883, rs5768709, rs6464375, rs708224, rs9502893 (one SNP identified in the Chinese GWAS is not polymorphic in Caucasians) in 1,299 PDAC cases and 2,884 controls. We also attempted stratified analysis considering the different stages of the disease and addressed the possible involvement of the selected SNPs on the survival of patients.

Results: None of the SNPs were significantly associated with PDAC risk if considering the overall population of the consortium. When stratifying for country of origin, we found that in the Polish subgroup, the G allele of rs372883 was statistically significantly associated with increased risk [OR, 6.40; 95% confidence interval (CI), 2.28–17.91]. However, the sample size of the subgroups was rather small; therefore, this result can be due to chance. None of the SNPs was associated with disease progression or survival.

Conclusions: None of the SNPs associated with PDAC risk in two Asian populations were convincingly associated with PDAC risk in individuals of European descent.

Impact: This study illustrates the importance of evaluation of PDAC risk markers across ethnic groups.

Cancer Epidemiol Biomarkers Prev; 22(2); 320–3. ©2012 AACR.

Authors’ Affiliations: ¹German Cancer Research Center (DKFZ), Heidelberg, Germany; ²Clinic for General, Visceral and Transplantation Surgery, University of Heidelberg, Heidelberg, Germany; ³Digestive and Liver Disease Unit, “Sapienza” University of Rome, Rome, Italy; ⁴Pancreas Biomedical Research Unit and the Liver Experimental Cancer Medicine Centre, National Institute for Health Research, Liverpool, United Kingdom; Departments of ⁵Digestive Tract Diseases and ⁶Hematology, Medical University, Lodz, Poland; ⁷Department of Digestive Diseases and Internal Medicine Sant’Orsola-Malpighi Hospital, Bologna, Italy; ⁸Laboratory of Biology, School of Medicine, University of Athens, Athens, Greece; ⁹University of Cambridge School of Clinical Medicine, Cambridge, ¹⁰Pancreatic Disease Epidemiology Unit, University of Oxford, Oxford, United Kingdom; ¹¹Azienda Ospedaliero Universitaria Meyer (A.O.U. Meyer) Ospedale Pediatrico, Florence, Italy; ¹²Department of Toxicogenomics, National Institute of Public Health, Prague, Czech Republic; ¹³Department of Molecular Biology of Cancer, Institute of Experimental Medicine, Academy of Science of the Czech Republic and First Faculty of Medicine, Charles University, Prague, Czech Republic; ¹⁴University of Pisa, Department of Biology, Pisa, Italy; and ¹⁵Medical Faculty Mannheim, Institute of Transfusion Medicine and Immunology, Heidelberg University, German Red Cross Blood Service Baden-Württemberg - Hessen, Mannheim, Germany

Note: D. Campa and C. Rizzato contributed equally to this work.

Corresponding Author: Federico Canzian, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, Heidelberg 69120, Germany. Phone: 49-6221-421791; Fax: 49-6221-421810; E-mail: f.canzian@dkfz.de
doi: 10.1158/1055-9965.EPI-12-1182 ©2012 American Association for Cancer Research.

Introduction

Pancreatic cancer mortality rates approach incidence rates (1). Due to the lack of sufficient risk factors, finding genetic variants associated with disease risk is of utmost importance. A genome-wide association study (GWAS) performed in a Caucasian population identified 4 loci associated with pancreatic cancer risk; 2 more showed a strong association only in samples from prospective cohorts but not in retrospective case-control series (2, 3). Two recent GWAS of pancreatic ductal adenocarcinoma (PDAC), conducted in Japan (4) and China (5), identified 8 novel loci affecting PDAC risk. We attempted to replicate these loci in a series of 1,299 PDAC and 2,884 healthy controls of European ancestry in the context of the PANcreatic Disease ReseArch (PANDoRA) consortium. We also attempted stratified analysis considering the different stages of the disease and addressed the possible involvement of the single-nucleotide polymorphisms (SNP) on the survival of patients.
Materials and Methods

Characteristics of the study population were described in detail elsewhere (6). A total of 1,299 PDAC cases and 2,884 controls were used in this study. We genotyped 4 SNPs identified by Wu (5) and 3 identified by Low (4). Genotyping was conducted using the KASPar SNP genotyping system (KBiosciences; ref. 7). Genotyping for British controls has been conducted in the context of a GWAS as described before (7).

Risk analysis was conducted by logistic regression for multivariate analyses to assess the main effects of the genetic polymorphism on pancreatic cancer risk using the same inheritance model reported by Wu and Low. The most common allele in the controls was assigned as the reference category. Survival analysis was conducted using HRs and 95% confidence intervals (CI) in Cox proportional hazard models. All analyses were adjusted for age, gender, tumor–node–metastasis (TNM) stage (for survival only), and nationality. We also conducted stratified analysis for risk and survival considering the various nationalities and the different stages as different strata. All analyses were conducted with STATA software (StataCorp).

Results

The 7 SNPs were genotyped in all cases and healthy controls. Relevant characteristics of the study population are given in Table 1. The average call rate was 95.86% (range, 93.93%–97.83%). Approximately 10% of the samples were analyzed in duplicate; the concordance rate of the genotypes was above 99%. The genotype distributions at all loci were in Hardy–Weinberg equilibrium in controls, with nonsignificant \( \chi^2 \) values (data not shown). The frequencies and distribution of the genotypes, the ORs and 95% CIs for the association with PDAC are shown in Table 2. Heterozygous AG carriers of rs1547374 were associated with increased risk (OR, 1.16; 95% CI, 1.00–1.35; \( P = 0.04 \)), whereas we observed a trend for rs5768709 and decreased risk (\( P_{\text{trend}} = 0.04 \)) and in heterozygous AG carriers of rs9502893 (OR, 0.84; 95% CI, 0.71–0.98; \( P = 0.03 \)). After Bonferroni correction, none of the above variants remained significant. Stratifying by nationality, we found that in the Polish subgroup, the G allele of rs372883 was significantly associated with increased risk (OR, 6.40; 95% CI, 2.28–17.91; \( P = 0.0004 \)). The sample size of the subgroup was rather small; there were 7 and 21 cases with AA or AG + GG genotypes, respectively, and 43 and 80 Polish controls with either AA or AG + GG genotypes. Therefore, this result has to be taken with caution. None of the other SNPs were significantly associated with PDAC risk, even when stratifying for tumor stage, or with patient survival, considering the correction for multiple testing. Finally, we observed no statistically significant association between the SNPs and survival when stratifying for nationality or tumor stage (Table 2).

<table>
<thead>
<tr>
<th>Nationality</th>
<th>Czech</th>
<th>German</th>
<th>Italian</th>
<th>Greek</th>
<th>Polish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>228</td>
<td>457</td>
<td>457</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>Controls</td>
<td>305</td>
<td>682</td>
<td>682</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td>Type of controls</td>
<td>Blood donors and hospitalized individuals</td>
<td>Blood donors</td>
<td>Blood donors and hospitalized individuals</td>
<td>Blood donors</td>
<td>Blood donors</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>146</td>
<td>307</td>
<td>312</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>146</td>
<td>307</td>
<td>312</td>
<td>75</td>
</tr>
<tr>
<td>Missing</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Age at diagnosis/recruitment</td>
<td>Median (25%–75%)</td>
<td>62.13 (49.46–75.19)</td>
<td>65.71 (57.70–71.74)</td>
<td>65.71 (57.46–75.36)</td>
<td>65.71 (57.46–75.36)</td>
</tr>
<tr>
<td>Survival days</td>
<td>Mean (SD)</td>
<td>289 (243)</td>
<td>478 (383)</td>
<td>478 (383)</td>
<td>478 (383)</td>
</tr>
<tr>
<td>Deaths</td>
<td>150</td>
<td>474</td>
<td>474</td>
<td>97</td>
<td>97</td>
</tr>
</tbody>
</table>

Table 1. Characteristics of patients and healthy controls included in this study
Discussion and Conclusion

We previously replicated the majority of loci identified by the first GWAS on pancreatic cancer in a subset of the cases and controls used in the present study (7). GWAS on PDAC risk in the Japanese (4) and Chinese population (5) yielded 3 new loci on chromosomes 6p25.3 (rs9502893, upstream of FOXI1), 12p11.21 (rs708224, in the second intron of BICD1), and 7q36.2 (rs6464375, in the first intron of DPP6) and 5 novel susceptibility loci at chromosomes 1q21.3 (rs372883, in the BACH1 gene), 5p13.1 (rs2255280, in the DAB2 gene), 21q22.3 (rs1547374, upstream of TFF1 gene), 22q13.32 (rs5768709), and 10q26.11 (rs12413624). The last 2 SNPs are not located in the immediate vicinity of any gene. We attempted to validate 7 of the novel hits in an independent cohort of different ethnicity. We did not analyze rs2255280 because it is monomorphic in Caucasians (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=2255280). Such validation is necessary because attempts to generalize genetic associations across ethnicities have had mixed results. Also, the incidence of pancreatic cancer is substantially different among populations of distinct ancestry (5), possibly reflecting differences in genetic susceptibility. We had more than 95% statistical power to detect the reported associations; nevertheless, we observed none. Our results highlight the genetic differences across human populations and illustrate the importance of evaluating PDAC risk markers across ethnic groups.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: D. Campa, C. Rizzato, J.P. Neoptolemos, M.W. Büchler, F. Canzian
Development of methodology: J.P. Neoptolemos, M.W. Büchler
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.S. Bauer, G. Capurso, E. Costello, R. Talar-Wojnarowska, K. Jamroziak, R. Pezzilli, M. Gazouli, K.-T. Khaw, T.J. Key,
References


Lack of Replication of Seven Pancreatic Cancer Susceptibility Loci Identified in Two Asian Populations

Daniele Campa, Cosmeri Rizzato, Andrea S. Bauer, et al.


Updated version  Access the most recent version of this article at: doi:10.1158/1055-9965.EPI-12-1182